

The University of Chicago

THE RESPIRATION OF NEURONES

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
1941

By
JANE PEARCE

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE AMERICAN JOURNAL OF PHYSIOLOGY, Vol. 136, March, 1942

The University of Chicago

THE RESPIRATION OF NEURONES

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
1941

By
JANE PEARCE



Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE AMERICAN JOURNAL OF PHYSIOLOGY, Vol. 136, March, 1942



Digitized by the Internet Archive
in 2026

THE RESPIRATION OF NEURONES¹

JANE PEARCE

From the Department of Physiology, University of Chicago

Accepted for publication December 19, 1941

A considerable literature now exists dealing with the gross respiration of the central nervous system, and evidence is accumulating on the respiration of structurally delimited regions (Gerard, 1938). Experiments of the latter type are mostly indirect, metabolic intensity being inferred from vascularity (Wolff, 1938; Craigie, 1941; Scharrer, 1939), resistance to anoxia (Sugar and Gerard, 1938; Kabat, Dennis and Baker, 1941; Fazekas, Alexander and Himwich, 1941; van Harreveld, 1941) or hypoglycemia (Tyler, 1940; Gellhorn, Packer and Feldman, 1940), concentration of oxidizing enzymes (Campbell, 1939), rates of reaction with special oxidation systems (ferric chloride, Gerard, 1938; ferricyanide, Michaelis and Quastel, 1941), etc. Also, in most such studies, the findings are at best applicable to considerable masses of cells mixed with other elements.

Many problems of neural function cry for more precise knowledge of the respiratory intensity per neurone or even neurone part. The present study, based on parallel measurement of oxygen consumption and cytoarchitecture of small bits of frog brain, is a step towards securing such knowledge.

METHODS. 1. *Tissue.* A frog was decapitated, the sides of the head snipped off, and the top of the brain case removed with forceps. The brain was carefully lifted on to a paraffin plate under a dissecting microscope and the meninges peeled off. Injured brains were discarded. A slice including the desired region was obtained by transections anterior and posterior to it, and then the particular region was cut out. A minimum of Ringer's solution (no substrate) was used. This was blotted off before weighing the tissue bit, which was remoistened in the capillary. Variations in QO_2 values (per gram moist tissue) are partly due to the difficulty of obtaining constant blotting, and proper weights, of tissue bits weighing but a milligram or two. From decapitation to completed dissection was never as much as five minutes, and respiration readings could be begun by another 5 to 10 minutes.

The structures chosen were of widely varied origin and architecture—the Anterior Olfactory Nucleus (AON), the Hippocampus (H), the Primordium Pallii (PP), the Ventral Hypothalamus (VT), and the Cerebellum (C) (fig. 1). AON composes the dorsal half of a slice obtained by a section at the junction of the olfactory bulbs and hemispheres and one a millimeter caudal. The cells in this nucleus receive fibers from the olfactory tract and send processes to the hemisphere. H constitutes the dorso-medial segment of the ring-shaped slice

¹ A preliminary report of this work appeared in This Journal, **129**: 445, 1940.

obtained from the middle third of the hemisphere. It is sharply delimited by gross landmarks, is large and relatively homogeneous, and is obtained with minimal sampling error. It represents the most highly organized brain region in the frog. PP is lateral to H, on the crest and lateral wall of the hemisphere with superficial white matter and a periventricular cell sheet. Since samples were often obtained by a simple horizontal slice from the top of the hemisphere, when speed was at a premium, this region was less precisely dissected than the others. VT bulges out from the ventral brain surface, the pituitary having been removed with the meninges. It is easily cut from a slice which includes the hypothalamus and contains considerable white as well as grey matter. C is the dorsal portion of the slice taken between the optic lobes and the 4th ventricle, and contains about half white matter. Since C is largely separate in situ, a minimum of damage is produced by cutting in this case.

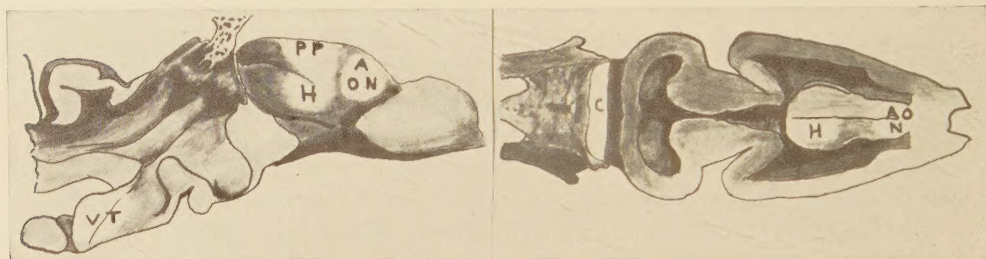


Fig. 1. Medial view of sagittal and view from above of horizontal section of the frog's brain, indicating the regions studied. Modified from "Handbuch der vergleichenden Anatomie der Wirbeltiere" and from Kappers-Huber-Crosby, Comparative Neurology, respectively.

2. *Respirometer*. The capillary respirometer (Gerard and Hartline, 1934) was used, with some modification, to measure oxygen consumption. To hasten temperature equilibrium, the instrument was placed vertically and the outer container filled with Ringer's solution except for 20 cc. of air trapped under inverted smaller tubes in it. Two thin-walled capillaries, about 2 sq. mm. cross section (later measured exactly in each case) and 200 c. mm. total volume, were carried on a lucite rod, which passed through the stopper of the outer vessel. Their upper ends were sealed with paraffin after inserting two filter paper strips and wetting them with isotonic KOH and H_2SO_4 respectively; tissue on a cellophane tray was placed through the other end of one, the second served as a control. All parts of the instrument were brought to temperature ($24^\circ C$) in the thermostat before the capillaries were mounted, and then placing the rod and stopper forced Ringer's solution a few millimeters into the capillaries, to serve as index fluid. Movements of the menisci, illuminated from behind with green light, were followed with an ocular micrometer in a long focus microscope. Some sticking of the menisci occurred and vibrating the holder with a buzzer for 5 sec. before each reading improved the regularity of readings.

One micrometer division corresponded to a volume of 0.0016 cu. mm., but ir-

regularities rendered short interval readings of little value. (See, however, further improvements by Tobias and Gerard, 1941.) Control and experimental tubes were read at alternate half minutes, and a sliding 3 min. average of the control was subtracted from a similar average of the tissue tube. In obtaining absolute QO_2 values for each tissue type, the final 30 min. readings of each run were averaged.

3. *Evaluation of cells.* Respiration values per cell are so far available only on naturally separate cell species, such as blood or sex cells or unicellulars. Counts on histological sections are usually impracticable because of irregular distribution and large numbers—about 100,000 per mgm. of brain. A possible solution is to macerate the brain sample after determining its respiration, and to count and measure the cell nuclei thus brought into homogeneous suspension.

The older histologists made frequent use of macerating solutions. Recently Crossman (1937) (also Stoneberg, 1939) has used 5 per cent citric acid to isolate muscle nuclei, and Isaacs (1937) and Farrar (1936) have removed connective tissue from bone marrow with serum. Zirkle (1928, et. seq.) has shown that, while basic fixatives destroy nuclear structures, acid ones preserve the nuclear chromatin. This is especially true of acetic acid which, because of rapid penetration, dominates in any mixture containing it. Further, he found that acid, per se, does not affect the size of cell nuclei, the usual shrinkage being due to dehydration.

In the present experiments the tissue bit was taken from the capillary, usually one hour after the decapitation, and gently triturated for 2 min. (in a 5 cc. glass test tube with a glass pestle ground to fit) in a measured volume—at least 20 times the tissue mass—of 25 per cent acetic acid in water. This dissolved the cytoplasm but left the nuclei intact in suspension. The suspension was further diluted with nine volumes of a 0.1 per cent solution of gentian violet in distilled water, to stain the nuclei. After thorough mixing, the diluted suspension was drawn into a red cell diluting pipette, mixed for an additional two minutes, and a drop placed in the hemocytometer counting chamber for counting. Two hundred to 1000 cells were counted in each sample. In calculating the cells per milligram (cells per cubic millimeter in the final suspension, times total volume in centimeters of acid and dye solutions, divided by sample weight in milligrams), the volume of the brain sample was neglected.

Several possible sources of error have been explored. That maceration and mixing were adequate was shown by the reproducibility of cell counts on separate drops of the same tissue suspension. Independent duplicate counts, two on each of three brain regions, gave a maximum variation between two drops of 7 per cent, an average of 4 per cent. A more severe check, testing in part the maceration and nuclear destruction as well as the reproducibility of the entire procedures of suspension and counting, was to compare cell counts on bilateral brain samples. Tests on three brain regions gave differences of 4, 17 and 21 per cent—well within the error of exact dissection and weighing of the tissue bits.

Further tests were made with frog's nucleated red cells, by comparing nuclear counts with each other and with cell counts made with Hayem's solution as

diluent. Blood was diluted 22 times with acetic acid (in the white cell counting pipette), the liquid expelled and "ground" for 2 min., diluted a further 10 times with gentian violet, and enumerated. Another sample of the same blood was merely diluted twice (1:22 and 1:10) with Hayem's solution. In three tests, on different bloods, the counts were, in thousands per cu. mm., 549 and 560, 582 and 596, and 749 and 741 in acetic acid and Hayem's solution respectively—an average deviation of 1.7 per cent. When one blood sample was directly diluted (1:200) with Hayem's solution and another portion weighed, "ground" with acetic acid, etc., the counts differed by as much as 14 per cent. These results prove that red cell nuclei are not destroyed by the maceration procedure used and that, except for the weighing of the sample, the whole method is valid to within 5 per cent. Since counts on bilateral regions agreed as well as the weigh-

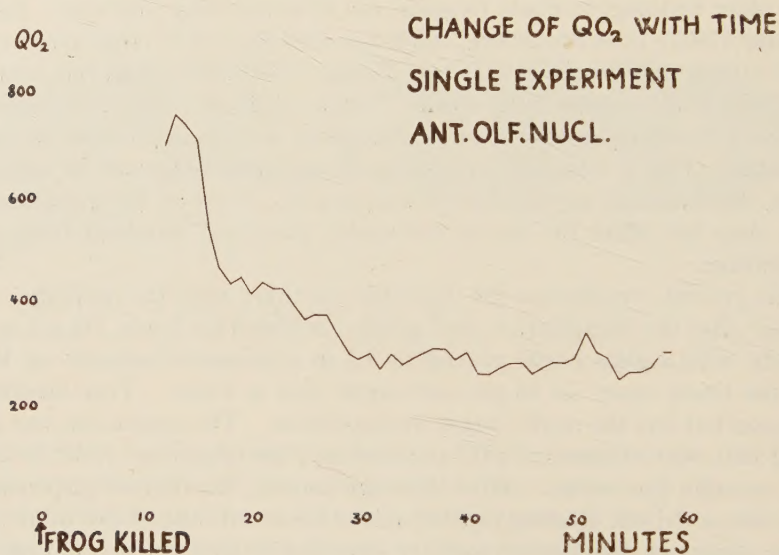


Fig. 2. Single experiment, illustrating reading variability

ing error permits, it is almost certain that no significant nuclear destruction occurred in these cells either.

The problem of what cells—neurones, neuroglia, endothelium, erythrocytes—are represented by the nuclei counted, and the related one of particular sizes and shapes of the nuclei from particular brain regions, will be considered later.

RESULTS. 1. *Respiration.* Seventy-seven oxygen consumption curves were obtained from the 5 brain regions and the sciatic nerve. Figure 2 reproduces a single curve and table 1 gives average values for each tissue. Decapitation is taken as zero time in all curves and averages. All brain regions, but not the sciatic, showed initially rising values, to a maximum at 15 to 20 min., and then a decelerating fall for the remainder of the hour.² For all quantitative com-

² The initially low readings are the result of an instrumental error, perhaps an initial lag in CO_2 absorption. When one bilateral brain bit is mounted in a capillary as usual, and the other after standing for 20 min. or more on the cellophane tray, the second shows the same

parisons, the average value of the fairly stable respiration of the last half-hour was used. This was but little greater than the value for the final 5 min.

The respiration of frog nerve (Gerard, 1930) and brain (Rosenberg, 1935) averages over 60 per cent higher in summer animals than in winter ones. Similar differences appeared in these experiments, although most of them were performed in the winter. The results with PP, however, were mostly obtained in the summer and cannot be directly compared with other values.

Results are summarized in table 1, which also includes the statistical estimate of their quantitative reliability. Winter values for brain QO_2 range from about 560 to 730 at the maximum and 390 to 470 in the final half-hour, about 8 times that of the sciatic; and summer values are higher. Yet even these are con-

TABLE 1
Mean respirations of various regions of frog nervous system

TISSUE	NUMBER OF FROGS USED	MEAN QO_2 * AT MAXIMUM	MEAN QO_2 FOR LAST 5 MIN. (1 HR. AFTER DECAPITA- TION)	MEAN QO_2 FOR LAST 30 MIN. AFTER DECAPITA- TION	σx † (FOR LAST 30 MIN.)	$\sigma \bar{x}$ ‡ (FOR LAST 30 MIN.)
Hippocampus.....	10	680	440	475	± 115	± 7
Cerebellum.....	10	725	355	400	± 130	± 8
Ant. Olf. Nucl.....	10	685	350	390	± 165	± 7
Hypothalamus.....	6	565	370	395	± 155	± 12
Sciatic nerve.....	10	90	60	65	± 30	± 2
Primordium Pallii.....	10	1055	610	675	± 735 §	± 50

* QO_2 = mm³ O₂/hr/gm wet weight.

† σx (standard deviation of the readings) = $\sqrt{\frac{\sum (x - \bar{x})^2}{N}}$, where x is a single reading, $\bar{x}$ the mean, N the number of readings. Over two-thirds of the readings normally fall in the range, $\bar{x} \pm \sigma x$.

‡ $\sigma \bar{x}$ (standard error of the mean) = $\frac{\sigma x}{\sqrt{N}}$. The chances of the observed $\bar{x}$ being more than $3\sigma \bar{x}$ from its "true" value are less than 3 in 1000.

§ The great variability of primordium pallii readings is due to sampling conditions. In many of these experiments a very rapid removal was the prime consideration and constant anatomical bits were not obtained.

low initial readings as had the first. Thus, in seven paired tests on PP (about 1.5 mgm. each) readings on the first samples averaged 1000 9.5 min. after decapitation, rose to 1400 4 min. later, and had fallen to 765 at 25 min. The second samples were mounted after half an hour and readings averaged 450 9 min. later, rose to 740 in another 5 min., and had fallen to 520 at the end of the run, one hour from decapitation. Clearly, except for the initial low period, the respiration of the second sample is a continuation of that of the first. The maximum values may, correspondingly, be somewhat excessive due to an "overshoot." This seems unlikely in view of the paired runs discussed and of the extensive evidence of a progressive fall of brain respiration after isolation (see Gerard, 1938), but is another reason for our using the later respiration values for quantitative evaluation. It is also worth noting that the high values during the first half-hour prove that oxygen diffusion into the tissue is not a limiting factor. This was further tested by the use of larger brain bits, which gave the same QO_2 values as smaller ones.

siderably above those of Rosenberg (1935), in the range 150 to 300, and of Ashford and Holmes (1931), 330 for intact and 250 for chopped brain; they are of the same order as those of Bass (1923), 415. Spring values for these same brain regions, obtained under similar but not identical conditions (Tobias and Gerard, 1941) average some 20 per cent higher. The maximal values here reported are probably more indicative of the *in vivo* rates than are the later ones; the lower values of other workers may be due to their working later after dissection and with whole brain, which contains a higher proportion of white matter than do our regions. The early high values are not due to injury—compare C, with only one cut surface, to AON with four—nor to an oxygen debt, for any possible inadequacy of oxygen could have lasted 3 to 5 min. at most. Conversely, the later low values are to be expected from substrate depletion and possible loss of a serum factor (Brookens, Ectors, and Gerard, 1936; Schaffer, Chang, and Gerard, 1935).

H has a higher respiration rate than the other brain regions, and is to be regarded as a "higher" center. Other direct *in vitro* respiration measurements on pigeon (Gavrilescu and Peters, 1931) and ox (Dixon and Meyer, 1936) (see also Quastel, 1939) offer interesting comparisons. The QO_2 of pigeon cerebellum, 1030, is 2.60 times that of the frog; and the cerebrum QO_2 , 1260, is 2.65 times that of the frog's hippocampus. Ox cerebral cortex, 1700, ammon's horn, 1260, and thalamus, 1170, QO_2 values are respectively 3.6, 2.7, and 2.9 times as great as those of the most equivalent frog structures. Evidence from anoxia (Sugar and Gerard, 1938; Hermann, et al., 1939) and hypoglycemia (Tyler and Ziskind, 1940) also indicates a parallelism between metabolic intensity of a brain region and its position along the neuraxis and in evolution.

2. *Cell population.* Figure 3 reproduces photomicrographs of characteristic fields obtained after maceration and staining of each of the brain regions. Although some debris is always present, the nuclei stand out as stained units containing chromatin structures and with a smooth unbroken boundary, the nuclear membrane, clearly visible. Only these were counted and measured; and the absence, or constancy, of any personal factor of selection is indicated by the degree of statistical reliability of the results.

It is apparent at once from figure 3 that the cell populations of the different brain regions are fairly unique for each, as judged by nuclear sizes and numbers. Similarly, the average cell count per hemocytometer square (0.1 mm.) was 105 for C, 70 for AON, and 27 for H. The results of the cell counts are presented in table 2. Despite considerable variation from brain to brain, the count for each region centers around its own characteristic value, which is significantly different from that of others.

For a more exact analysis, and to distinguish between kinds of cells, nuclear dimensions were measured with an ocular micrometer. The greatest diameter and the maximum width at right angles to this were obtained on 100 consecutive nuclei, as located across the counting chamber, of each preparation. Since 10 such preparations of AON, H, and C were studied, a composite picture of 1000 nuclei was obtained for each of these regions.

Nuclei of blood cells were obtained by applying the maceration technique to whole blood, and the dimensions of 300 nuclei, from 3 samples, measured as above. To identify endothelial cell nuclei in the brain bits, the trituration in acid was stopped early, in half a minute, and the imperfectly macerated tissue then stained as usual. Scraps of capillary endothelium were thus preserved and their nuclei identified. Three hundred of these were measured. The dis-

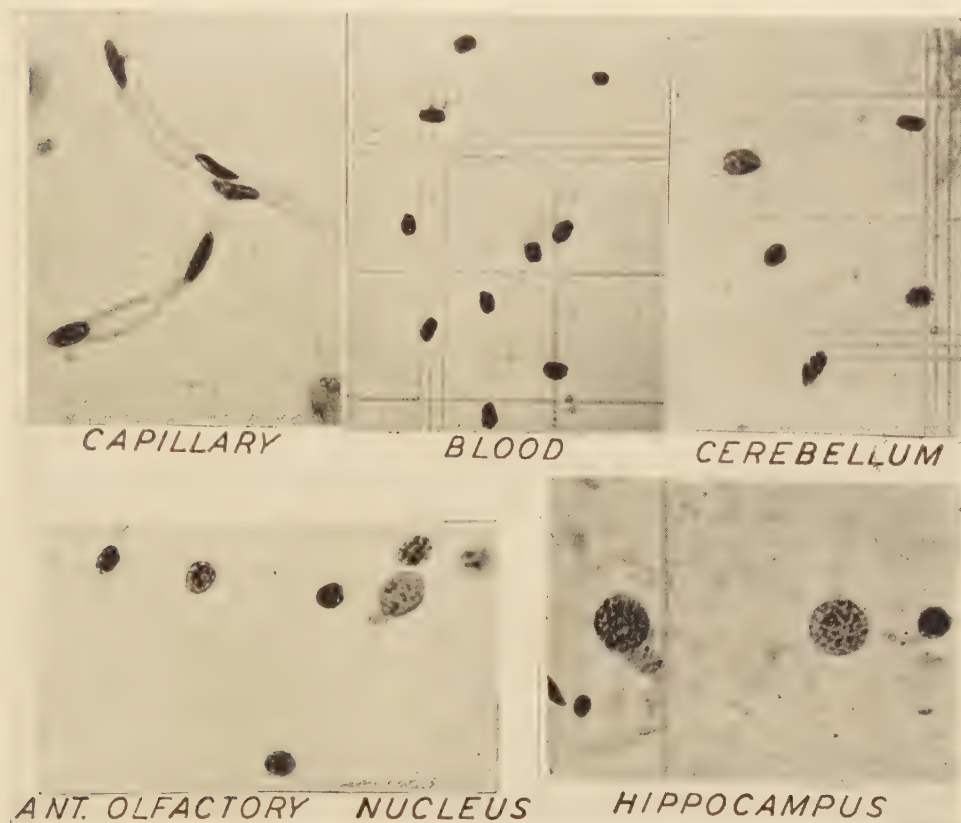


Fig. 3. Photomicrographs of characteristic fields obtained after maceration and staining of nuclei. Five-hundredths millimeter between squares on the R.B.C. field gives magnification.

crimination of glial and neurone nuclei was not satisfactory.³ These cell types will, therefore, be considered together.

Although fixation shrinkage and distortion would not affect relative measurements of the different cell nuclei, absolute values might be seriously off. To check this, red cell nuclei were measured after treatment with Hayem's solution and with acetic-gentian violet and the results compared. Table 3 shows clearly

³ Using toluidine blue, instead of gentian violet, Doctor Tobias finds the glial nuclei to stain with a more greenish tinge than those of neurones, but the conditions for obtaining this differentiation have not been worked out.

TABLE 2
Total cells per milligram for each tissue (multiply all figures by 10³)

EXPERIMENT NUMBER	BLOOD	HIPPOCAMPUS*	CEREBELLUM	ANTERIOR OLFACTORY NUCLEUS	HYPOTHALAMUS	WHOLE CEN- TRAL NERVOUS SYSTEM
1	640	60	215	175	145	61
2	560	49	205	115	100	
3	550	46	250	190	175	
4	550	48	260	90	145	
5	580	49	235	90	155	
6	595	42	260	135		
7	750	55	315	75		
8	740	45	165	145		
9	690	62	200	105		
10	610	67	170	120		
$\bar{x}$	627	52	228	124	143	
σx	69	8	43	36	25	
$\sigma \bar{x}$	± 26	± 3	± 16	± 13	± 14	

* The more consistent figures for hippocampus than for other regions are undoubtedly the consequence of better sampling. Because of the few large cells, more drops of suspension were counted. Had a similarly extensive sampling been used in the other cases a corresponding improvement in reliability should have resulted.

TABLE 3
Comparison of nuclear dimensions in maceration fluid and in Hayem's solution

	METHOD OF PREPARATION	
	Acid and gentian violet	Hayem's solution
	100 cells	50 cells
Surface:		
Average.....	144	150
σx	29	33
$\sigma \bar{x}$	± 2.9	± 4.6
P	0.12	
Volume:		
Average.....	135	140
σx	41	24
$\sigma \bar{x}$	± 4.1	± 3.4
P	1.4	

Statistical analysis of the measurements on red cell nuclei prepared in Hayem's and in acid and gentian violet. Surface was calculated, for convenience, by the formula of $S = \pi dl$, where d and l are the short and long diameters respectively. Volume is calculated by the formula $V = \frac{\pi}{6} d^2 l$. P is the probability that the two samples are from the same universe. As P is much greater than 0.05 this identity can be taken as established.

that nuclei prepared by both methods fall into a single population; that is, the two treatments are equivalent. This finding is in agreement with Zirkle's results.

A further test was made by comparing nuclear dimensions of bilateral bits from the same brain. In figure 4 the abscissa represents the long nuclear diameter, the ordinate the short one, and each nucleus is represented by a dash within the one micron square to which its measurements relegate it. Since nuclei from one side are shown as horizontal dashes and those from the other as



Fig. 4. Chart showing distribution of nuclear dimensions from like bilateral samples of one brain. The long and short diameters are indicated in microns. Each dash represents one nucleus; horizontal ones from one side of the brain, vertical ones from the other.

vertical ones, the crosses at once indicate the high degree of coincidence of the two sets. A similar amount of overlap was obtained when some nuclei in a given suspension were measured at once after preparation and others 24 hours later. Finally, measurements made on nuclei in fixed (formol acetic) and stained frog brain sections were compared with those on macerated preparations (we are indebted to Dr. M. L. Silver for values on one such set).

Rough long and short nuclear diameters, in sections and suspension respectively, are: red cells, 8 x 4 and 10 x 5; endothelial cells, 12 x 8 and 15 x 7; H, 12 x 12 and 17 x 17; C, 8 x 6 and 10 x 10; AON 10 x 6 and 15 x 10. Blood and

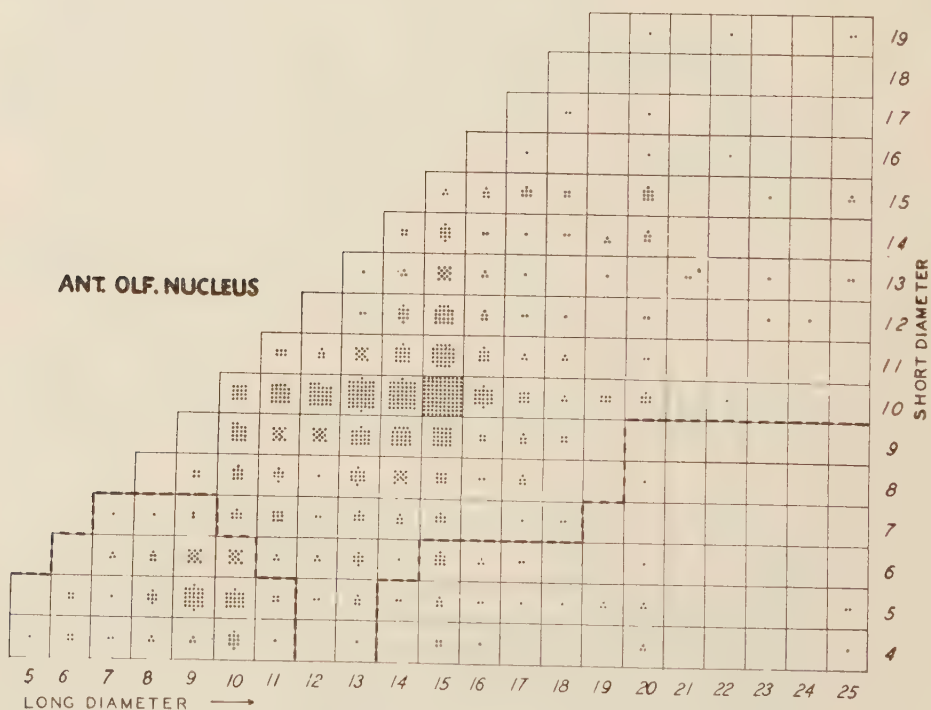
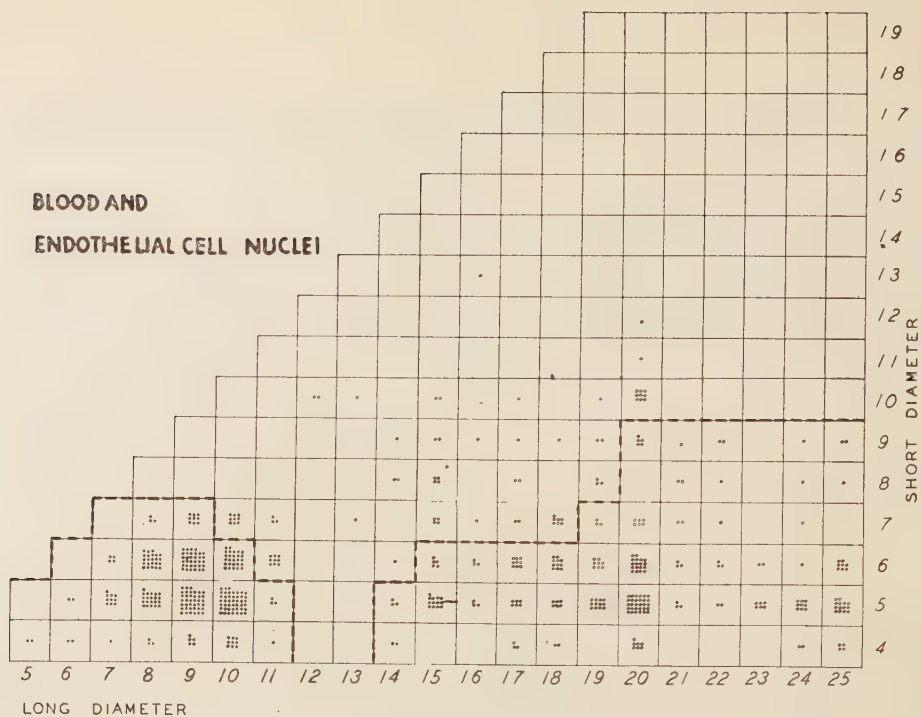


Fig. 5. Charts showing the distribution of nuclear dimensions in various tissues. Each dot represents one cell nucleus of the indicated dimensions in microns. The blood and endothelial cells were measured in separate preparations. The dotted lines in the brain charts are drawn to include 90 per cent of these non-neural cells.

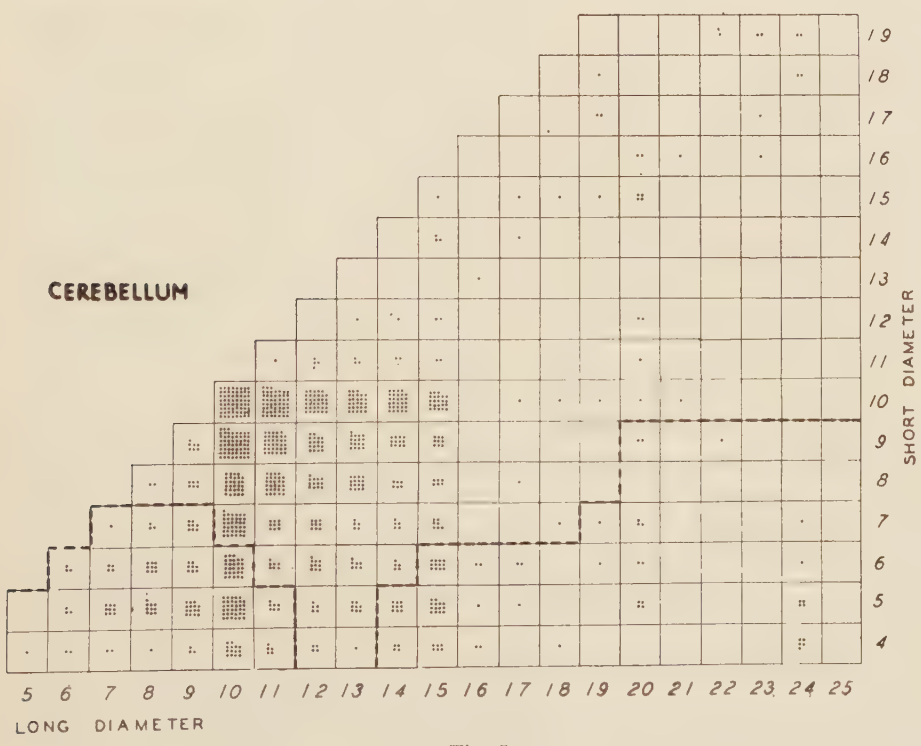
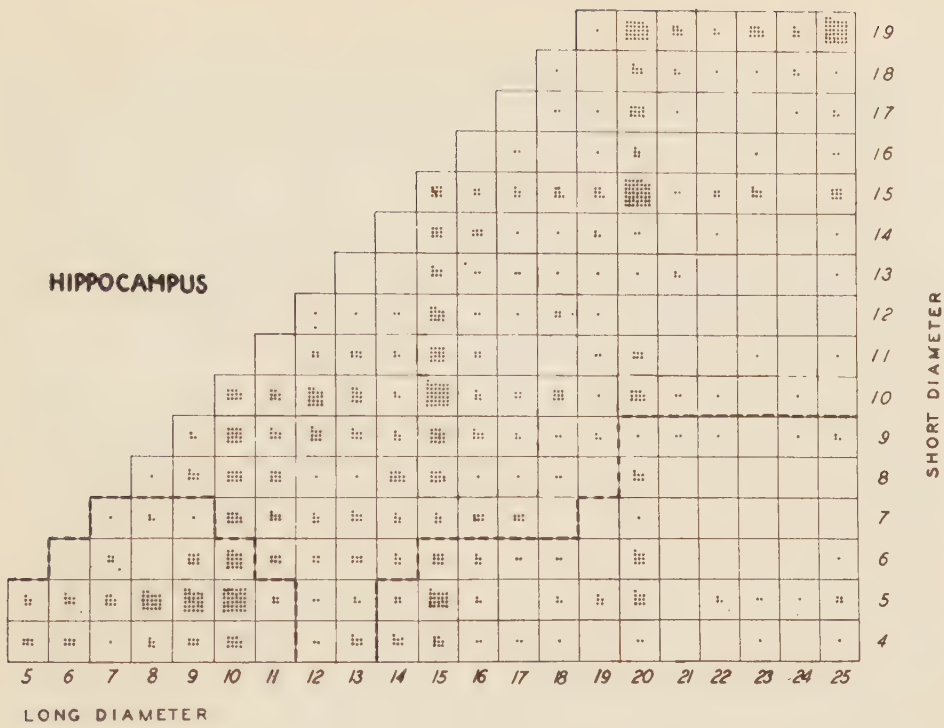


Fig. 5

capillary cell nuclei show fair agreement, the fixed preparations being somewhat larger, while the discrepancy with nuclei of neural elements is greater. Since technical errors also appear in section measurements and since a large sampling error is probable (only a few dozen nuclei were measured), it is hard to evaluate this divergence; but obviously the absolute dimensions we have used are open to some question. For a doubling of radii, values per unit surface would be low in absolute units by a factor of four; values per unit volume, by a factor of eight. Fortunately, even if all neural nuclei are swollen in our suspensions, the various relationships will still hold, as between the different brain regions, if the swelling be proportional to true size—a reasonable assumption.

Charts showing the distribution of nuclear dimensions for the three intensively studied brain regions and for erythrocytes and endothelial cells are presented in figure 5. That these differ is patent. Cerebellar nuclei are small and rather homogeneous, centering about $18 \times 10 \text{ m}\mu$; those of AON are more variable and center about $10 \times 15 \text{ m}\mu$; while those of H are quite scattered and run to much larger dimensions.

It is necessary to correct the brain regions by excluding blood and endothelial nuclei in order to deal only with neural elements. This is difficult because of some overlap in dimensions of nuclei of these different types; but it is none-the-less possible to make an adequate separation. An area was blocked off on the nuclear dimension charts of blood and of endothelial cells so as to include 90 per cent of all nuclei in each case. The same areas were then marked on the charts for each neural region and the nuclei in them excluded from the final count. Thus, presumably, the neural count includes some 10 per cent each of the other cell types and excludes an unknown portion of neural elements. Actually, the overlap of dimensions is small enough and the percentage of non-neural nuclei in the suspension low enough so that varying the areas subtracted, to include 100 per cent or 80 per cent of the non-neural nuclei instead of the usual 90 per cent, alters the neural cell count by only 2 to 3 per cent. The error in evaluating blood and endothelial cells is, of course, greater. Further refinements should, however, make it possible to estimate the relative capillary length and the blood supply⁴ for each brain region from such nuclear studies. Table 4 gives the cell relations as obtained by our present procedure.

3. *Nuclear dimensions.* Aside from their value in distinguishing cell types, the nuclear diameters permit calculation of other nuclear dimensions. Assuming an ellipsoid shape of the nucleus, the third, unmeasured, diameter was taken equal to the shorter measured one. Nuclear volume was then obtained from the formula $V = \frac{\pi}{6} d^2 l$ (d = short diameters, l = long one). Nuclear surface was calculated, for convenience, in terms of a cylinder, of length $l - d$, capped by hemispheres, of diameter d . $S = \pi dl$. For other comparisons, nuclear volume

⁴ For example, from the known red cell count and the average number of red cell nuclei per milligram of brain tissue, it can be calculated that blood constitutes over 2 per cent of the brain volume, despite the free hemorrhage permitted on decapitation. Compare with the value of 8.3 per cent for the dog's brain (Weil, Zeiss and Cleveland, 1931).

squared (V^2) and to the three halves power ($V^{3/2}$) have also been calculated. These four values were calculated for each set of nuclear dimensions. To obtain, say, total nuclear volume, ΣV , per cubic millimeter of any brain region, the

TABLE 4

Percentage and absolute numbers per milligram of red blood cells, capillary cells, and nerve and glia cells in each tissue. (Calculated from fig. 5)

TISSUE	NERVE AND GLIA CELLS	RED BLOOD CELLS	CAPILLARY CELLS	TOTAL CELLS PER MGM.	RED BLOOD CELLS PER MGM.	CAPILLARY CELLS PER MGM.	NERVE AND GLIA CELLS PER MGM.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>				
Hippocampus.....	67	17.5	15.5	52,300	9,000	8,000	35,000
Ant. Olf. Nuc.....	80.5	13	6.5	123,400	15,000	8,000	100,000
Cerebellum.....	70	19	11	227,600	43,500	25,000	160,000

TABLE 5

Calculated functions of the nuclear dimensions of nerve and glia cells

FUNCTION	CELLS	HIPPOCAMPUS	CEREBELLUM	ANT. OLF. NUCL.
Average function per cell, in microns				
Nuclear surface (μ^2).....	Total	405 $\pm$ 13*	275 $\pm$ 4	380 $\pm$ 4
	"Nerve"	505	315	420
Nuclear volume (μ^3).....	Total	935 $\pm$ 45	395 $\pm$ 14	585 $\pm$ 14
	"Nerve"	1290	495	685
(Nuclear volume) ² (μ^4) ²	Total	2,950,000 $\pm$ 210,000	325,000 $\pm$ 40,000	539,000 $\pm$ 35,000
	"Nerve"	4,350,000	445,000	654,000
(Nuclear volume) ^{3/2} (μ^3) ^{3/2}	Total	45,500 $\pm$ 2,900	10,000 $\pm$ 720	17,000 $\pm$ 700
	"Nerve"	66,000	13,500	20,000
Average function per milligram of tissue, in microns ($\times 10^{-6}$)				
Nuclear surface.....	Total	21.5 $\pm$ 1.4	62.5 $\pm$ 4.5	46.5 $\pm$ 5.0
	"Nerve"	17.5	50.5	41
Nuclear volume.....	Total	48.5 $\pm$ 3.6	89.5 $\pm$ 7.0	71.5 $\pm$ 7.9
	"Nerve"	45	78.5	68.5
(Nuclear volume) ²	Total	155,000 $\pm$ 14,000	74,000 $\pm$ 11,000	66,000 $\pm$ 8,000
	"Nerve"	155,000	71,000	65,500
(Nuclear volume) ^{3/2}	Total	2,400 $\pm$ 200	2,300 $\pm$ 230	2,100 $\pm$ 240
	"Nerve"	2,300	2,200	2,000

* These represent the standard errors of the means. For the average function per cell for each tissue these were calculated as described in table 1. For the total surface, volume, etc. per milligram the standard errors were calculated by the formula for a product. For example, if the average nuclear volume per cell in a given tissue is $V \pm \sigma V$, and the number of cells per milligram is $N \pm \sigma N$, the average nuclear volume per milligram is

$$NV \pm \sigma NV, \quad \sigma NV = \sqrt{V^2(\sigma N)^2 + N^2(\sigma V)^2}$$

volumes corresponding to nuclei in each dimension group were summed in numbers proportional to (by the factor, total nuclei per cubic millimeter over 1000) the nuclei found in that dimension area in the plot (fig. 5) of the desired

brain region. The average nuclear volume was then obtained by dividing this total volume per cubic millimeter by the cells per cubic millimeter. Such total and average nuclear functions were calculated, for each of the brain regions intensively studied, on the basis of all cells (1000 for each region) measured in ten experiments. Similar calculations were also made for the neural elements only, after excluding blood and capillary cells. The results are given in table 5.

DISCUSSION. Bok (1936) has published exhaustive quantitative studies of the neurone population of several areas of the human cortex, supplemented by some on primate cord (1934). He reports many striking relationships between nuclear parameters and other cell dimensions and distributions. Thus, for various cytoarchitectonic regions, with few large or many small neurones, total nuclear volume squared (ΣV^2 , the sum of V^2 of all individual nuclei) is constant. Strictly proportional to V^2 is the cytoplasmic mass of each neurone (the perikaryon volume, equal to the volume of cell body after excluding nucleus and processes); and also the "territory" governed by each neurone—the volume occupied by its dendrites and by the terminals of other cells connecting with these—which is 35 times the perikaryon volume. The total length of all nerve processes is constant per unit brain volume, and so proportional to ΣV^2 ; and the dendrite length of each neurone varies as its own V^2 , so that total dendrite length is also constant per unit brain volume. Thus, all brain regions have a constant proportion of perikarya, dendrites, and incoming fibers, but each is characterized by the size of the individual cell units—and all these quantities vary directly as V^2 or ΣV^2 .

The surface area of the perikaryon is also a function of its nuclear volume, but in this case directly proportional to V ; whereas the total volume of each cell body (nucleus and cytoplasm) is proportional to $V^{3/2}$. Thus, per unit volume of grey matter, cytoplasmic mass, dendrite length, and total nerve fiber length vary as ΣV^2 ; perikaryon surface varies as ΣV ; and protoplasmic volume as $\Sigma V^{3/2}$.

This study covers three widely differing brain regions in the frog, each extending from pia to ventricle and so presumably including the full "territories" of the contained neurones. An inverse relation between size and number of cells in these regions is at once apparent, but ΣV^2 is not a constant. Either, therefore, such attributes as cytoplasmic mass and dendrite length are not proportional to V^2 in frog neurones or their total values are not constant per brain mass. ΣV , and presumably perikaryon surface, is also not constant from one brain region to another. $\Sigma V^{3/2}$ is, however, fairly constant in these frog brain regions and so, perhaps, is the volume of cell protoplasm.

Interestingly, the fraction of brain volume occupied by cell bodies decreases—and that available for synaptic field increases—from frog to man. Thus, Donaldson (1911) estimated cell bodies to occupy 2 per cent of the human brain and 3 to 5 per cent of the rat brain, and Bok finds almost 3 per cent of human cortex occupied by cell cytoplasm; while we find 6 to 8 per cent occupied by nuclei alone in the frog. Truszkowski's finding (1928), that the ratio of purine to total nitrogen is 50 per cent higher in frogs than in mammals, suggests a similarly greater nuclear mass in frog tissues as a whole.

Before correlating the respiration data with the cytological findings, some consideration of the oxygen consumption of non-neural cells is necessary. Measurements of the respiration of frog blood gave an average QO_2 of 36; and of urostyle bone marrow (as somewhat representative of endothelial elements) a QO_2 of 74 for a preparation with 1.6×10^5 cells per milligram. The average brain sample contained 0.03 mgm. of blood and 10^4 endothelial cells per milligram. These would thus account, respectively, for 1 and 5 cm. O_2 per hour per gram of brain—entirely negligible amounts in comparison with the measured QO_2 values of 400 to 500. It is also to be noted that, since respiration and cell measurements are available on the same brain bits, weighing and other sampling errors will cancel rather than cumulate when oxygen consumption is calculated in terms of cells rather than of total mass.

TABLE 6

Respiration as a function of the parameters of the cell nuclei of each brain region

FUNCTION	CELLS	HIPPOCAMPUS	CEREBELLUM	ANT. OLF. NUC.
$QO_2/\text{cu.mm.}O_2/\text{gm.}/\text{hr.}$		475 ± 7	400 ± 8	390 ± 7
O_2 uptake per cell (cu.mm. per hr. $\times 10^{-6}$).....	Total	9.1	1.7	3.1
	"Nerve"	13.5	2.5	3.9
QO_2 per unit surface (cu.mm. $O_2/\mu^2/\text{hr.} \times 10^{-9}$).....	Total	$22.3 \pm 1.8^*$	6.4 ± 0.5	8.3 ± 0.9
	"Nerve"	26.8	7.9	9.3
QO_2 per unit volume (cu.mm. $O_2/\mu^3/\text{hr.} \times 10^{-9}$).....	Total	9.7 ± 0.7	4.5 ± 0.4	5.4 ± 0.6
	"Nerve"	10.5	5.1	5.7
QO_2 per unit (volume) 2 (cu.mm. $O_2/(\mu^3)^2/\text{hr.} \times 10^{-12}$).....	Total	3.1 ± 0.3	5.4 ± 0.8	5.8 ± 0.7
	"Nerve"	3.1	5.6	6.0
QO_2 per unit (volume) $^{\frac{2}{3}}$ (cu.mm. $O_2/(\mu^3)^{\frac{2}{3}}/\text{hr.} \times 10^{-12}$).....	Total	200 ± 17	175 ± 18	185 ± 22
	"Nerve"	205	195	195

* The standard error, of the respiration per particular tissue dimension, is calculated on the basis of the statistics of the component variables by a formula for the standard error of ratios (see J. B. Scarborough, *Numerical Mathematical Analysis*, The Johns Hopkins Press, Baltimore, 1930).

$$\sigma \left(\frac{\bar{x}}{\bar{y}\bar{z}} \right) = \frac{\bar{x}}{\bar{y}\bar{z}} \sqrt{\left(\frac{\sigma \bar{x}}{\bar{x}} \right)^2 + \left(\frac{\sigma \bar{y}}{\bar{y}} \right)^2 + \left(\frac{\sigma \bar{z}}{\bar{z}} \right)^2}$$

Where $\bar{x}$, $\bar{y}$ and $\bar{z}$ are the means respectively for respiration, cell number, and nuclear volume. We are indebted to Miss Eleanor Gish for assistance with all of the statistical calculations in this paper.

Table 6 gives the respiration as a function of various cell parameters for each brain region. It is obvious at a glance that, while respiration per milligram is but little greater in H than in AON or C, respiration per cell is 3 to 5 times as great—H having few large cells—while respiration per mass of cell protoplasm ($\Sigma V^{3/2}$) is essentially alike in all. Further study shows that, assuming Bok's relations, the respirations of different brain masses are alike only when expressed as a function of the total nerve cell protoplasm contained in each, and not when correlated with the summated: volumes of nuclei or of cytoplasm alone, surfaces of nuclei or of cell bodies, lengths or surfaces of cell processes. Thus these findings, somewhat disappointingly, point to the whole protoplasmic mass rather than to surface membranes as determining the magnitude of the resting metabolism and the energy requirement of neurones.

SUMMARY

1. Bits of frog brain tissue, about 1 mgm., were dissected from widely different structural regions: anterior olfactory nucleus (AON), primordium pallii (PP), hippocampus (H), ventral hypothalamus (VT), and cerebellum (C).

2. Respiration of these bits, as of nerve, was determined in a capillary respirometer; and the tissue was then macerated and the nuclei of nerve cells (and glia, capillary endothelium, and red blood cells) counted and measured. The method of cell evaluation is intrinsically valid to 5 per cent.

3. QO_2 values, up to 700, are higher than most of those previously reported for frog and average about 40 per cent of those for mammalian brain. In order of descending QO_2 , the series is C, H, AON, (PP), VT at first; with C falling off faster than other regions.

4. Nerve cells per milligram vary from 35,000 large neurones in H to 160,000 small ones in C, with a characteristic size population in each region. The total cell volume is roughly the same for all regions.

5. These data make it possible to express oxygen consumption as a function of the total neurones and, assuming the morphologic relations described by Bok, of the total neurite surface, perikaryon surface, process surface, nuclear surface, nuclear volume, perikaryon volume and total protoplasm volume. All these functions are analyzed statistically for valid correlations.

6. The respiration of various brain regions is constantly related to the mass of protoplasm, but not to the surface area, of the neural elements present.

REFERENCES

- ASHFORD, C. A. AND E. G. HOLMES. *Biochem. J.* **25**: 2028, 1931.
 BASS, E. *Ztschr. Biol.* **78**: 161, 1923.
 BOK, S. T. *Kon. Akad. Wet., Verhand. (Tweede Sectie)* **35**: 1, 1936.
 Psychiat. neurol. Bl., Amsterdam **38**: 54, 1934.
 BROOKENS, N. L., L. ECTORS AND R. W. GERARD. *This Journal* **116**: 16, 1936.
 CAMPBELL, A. C. F. *Arch. Neurol. and Psychiat.* **41**: 223, 1939.
 CRAIGIE, E. H. *J. Comp. Neurol.* **64**: 453, 1938.
 J. Morph. **69**: 263, 1941.
 CROSSMAN, G. *Science* **85**: 250, 1937.
 DIXON, T. E. AND A. MEYER. *Biochem. J.* **30**: 1577, 1936.
 DONALDSON, H. H. *J. Nerv. Ment. Dis.*, **38**: 257, 1911.
 FARRAR, G. E., JR. *This Journal* **117**: 662, 1936.
 FAZEKAS, J. F., A. D. ALEXANDER AND H. E. HIMWICH. *This Journal* **134**: 281, 1941.
 GAVRILESCU, N. AND R. A. PETERS. *Biochem. J.* **25**: 1397, 1931.
 GELLHORN, E., A. PACKER AND J. FELDMAN. *This Journal* **130**: 261, 1940.
 GERARD, R. W. *Proc. Soc. Exper. Biol. and Med.* **27**: 1052, 1930.
 Assoc. Res. Nerv. and Ment. Dis. **18**: 316, 1938.
 GERARD, R. W. AND H. K. HARTLINE. *J. Cell. Comp. Physiol.* **4**: 141, 1934.
 HERMANN, H., F. JOURDAN AND P. SEDALLIAN. *C. R. Soc. Biol.* **130**: 370, 1939.
 HEYMANS, C., J. J. BOUCKAERT, F. JOURDAN, S. J. G. NOWAK AND S. FARBER. *Arch. Neurol. Psychiat., Chicago* **38**: 304, 1937.
 HOAGLAND, H., H. E. HIMWICH, E. CAMPBELL, J. F. FAZEKAS AND A. HADIDIAN. *J. Neurophysiol.* **2**: 276, 1939.
 ISAACS, R. *Trans. Assoc. Am. Physicians* **50**: 249, 1935.
 Am. J. Med. Sciences **193**: 181, 1937.

- KABAT, H., C. DENNIS AND A. B. BAKER. This Journal **132**: 737, 1941.
- MICHAELIS, H. AND J. H. QUASTEL. Biochem. J. **35**: 518, 1941.
- QUASTEL, J. H. Physiol. Rev. **19**: 135, 1939.
- ROSENBERG, J. Arch. Int. Physiol. **41**: 434, 1935.
- SCHARRER, E. Anat. Rec. **75**: 319, 1939.
- SHAFFER, M., T. H. CHANG AND R. W. GERARD. This Journal **111**: 697, 1935.
- STONEBERG, C. A. J. Biol. Chem. **129**: 189, 1939.
- SUGAR, O. AND R. W. GERARD. J. Neurophysiol. **1**: 558, 1938.
- TOBIAS, J. AND R. W. GERARD. Proc. Soc. Exper. Biol. and Med. **47**: 531, 1941.
- TRUSZKOWSKI, R. Biochem. J. **22**: 198, 1928.
- TYLER, D. B. This Journal **131**: 554, 1940.
- VAN HARREVELD, A. This Journal **133**: 572, 1941.
- WEIL, A., F. R. ZEISS AND D. A. CLEVELAND. This Journal, **98**: 365, 1931.
- WOLFF, H. G. Physiol. Rev. **16**: 545, 1936.
- Res. Public Soc. Res. Nerv. Ment. Dis. **18**: 29, 1938.
- ZIRKLE, C. Protoplasma **4**: 201, 1928; **5**: 511, 1929; **18**: 90, 1933.
- ZISKIND, E. AND D. B. TYLER. Proc. Soc. Exper. Biol. and Med. **43**: 734, 1940.

